

What is claimed is:

1. A theta defensin peptide, or a functional fragment thereof, said theta defensin peptide having antimicrobial activity.

5 2. The theta defensin peptide of claim 1, or a functional fragment thereof, having the amino acid sequence:

Xaa1-Xaa2-Xaa3-Xaa4-Xaa5-Xaa1-Xaa6-Xaa4-Xaa4-Xaa1-Xaa1-Xaa6-Xaa4-Xaa5-Xaa1-Xaa3-Xaa7-Xaa8,

10 wherein: Xaa1 independently is an aliphatic amino acid;
 Xaa2 is an aromatic amino acid;
 Xaa3 is Cys or Trp;
 Xaa4 independently is Arg or Lys;
 Xaa5 is Cys or Trp;
15 Xaa6 is Cys or Trp;
 Xaa7 is Thr or Ser; and
 Xaa8 is Arg or Lys.

3. The theta defensin peptide of claim 2, or a functional fragment thereof, having the amino acid sequence:

Xaa1-Xaa2-Xaa3-Xaa4-Xaa5-Xaa1-Xaa6-Xaa4-Xaa4-Xaa1-Xaa1-
5 Xaa6-Xaa4-Xaa5-Xaa1-Xaa3-Xaa7-Xaa8,

wherein: Xaa1 independently is Gly, Ile, Leu, Val or Ala;

Xaa2 is Phe, Trp or Tyr;

Xaa3 is Cys or Trp;

10 Xaa4 independently is Arg or Lys;

Xaa5 is Cys or Trp;

Xaa6 is Cys or Trp;

Xaa7 is Thr or Ser; and

Xaa8 is Arg or Lys.

15 4. The theta defensin peptide of claim 3, having the amino acid sequence:

Gly-Phe-Cys-Arg-Cys-Leu-Cys-Arg-Arg-Gly-Val-Cys-Arg-Cys-Ile-Cys-Thr-Arg (SEQ ID NO:1).

20 5. The theta defensin peptide of claim 3, wherein Xaa1 is linked through a peptide bond to Xaa8.

6. The theta defensin peptide of claim 3, wherein an intrachain crosslink is formed between two amino acids selected from the group consisting of:

Xaa3 at position 3 and Xaa3 at position 16;

25 Xaa5 at position 5 and Xaa5 at position 14; and

Xaa6 at position 7 and Xaa6 at position 12.

7. The theta defensin peptide of claim 6, wherein an intrachain crosslink is formed between:

Xaa3 at position 3 and Xaa3 at position 16;
Xaa5 at position 5 and Xaa5 at position 14; and
5 Xaa6 at position 7 and Xaa6 at position 12.

8. The theta defensin peptide of claim 6, wherein Xaa1 is linked through a peptide bond to Xaa8.

9. The theta defensin analog of claim 6, wherein said intrachain crosslink is a disulfide
10 crosslink.

10. The theta defensin of claim 6, wherein said intrachain crosslink is a di-tryptophan crosslink.

11. The theta defensin of claim 6, wherein said intrachain crosslink is a lanthionine crosslink.

15 12. The theta defensin peptide of claim 8, having the amino acid sequence:

Gly-Phe-Cys-Arg-Cys-Leu-Cys-Arg-Arg-Gly-Val-Cys-Arg-Cys-Ile-Cys-Thr-Arg (SEQ ID NO:1).

13. The theta defensin of claim 12, comprising
20 three disulfide crosslinks consisting of

Xaa3 at position 3 and Xaa3 at position 16;
Xaa5 at position 5 and Xaa5 at position 14; and
Xaa6 at position 7 and Xaa6 at position 12.

14. The theta defensin of claim 1, comprising the amino acid sequence

Arg-Cys-Ile-Cys-Thr-Arg-Gly-Phe-Cys (SEQ ID NO:18) or
Arg-Cys-Leu-Cys-Arg-Arg-Gly-Val-Cys (SEQ ID NO:20).

5 15. The theta defensin of claim 14, having the amino acid sequence:

Gly-Phe-Cys-Arg-Cys-Ile-Cys-Thr-Arg-Gly-Phe-Cys-Arg-Cys-Ile-Cys-Thr-Arg (SEQ ID NO:30).

10 16. The theta defensin of claim 15, wherein the Gly at position 1 is linked through a peptide bond to the Arg at position 18.

17. The theta defensin of claim 16, wherein an intrachain crosslink is formed between two amino acids selected from the group consisting of:

15 Cys at position 3 and Cys at position 16;
Cys at position 5 and Cys at position 14; and
Cys at position 7 and Cys at position 12.

18. The theta defensin of claim 17, wherein a disulfide bond is formed between:

20 Cys at position 3 and Cys at position 16;
Cys at position 5 and Cys at position 14; and
Cys at position 7 and Cys at position 12.

19. The theta defensin of claim 14, having the amino acid sequence:

Gly-Val-Cys-Arg-Cys-Leu-Cys-Arg-Arg-Gly-Val-Cys-Arg-Cys-Leu-Cys-Arg-Arg (SEQ ID NO:31).

5 20. The theta defensin of claim 19, wherein the Gly at position 1 is linked through a peptide bond to the Arg at position 18.

 21. The theta defensin of claim 20, wherein an intrachain crosslink is formed between two amino acids
10 selected from the group consisting of:

Cys at position 3 and Cys at position 16;
Cys at position 5 and Cys at position 14; and
Cys at position 7 and Cys at position 12.

 22. The theta defensin of claim 21, wherein a
15 disulfide bond is formed between:

Cys at position 3 and Cys at position 16;
Cys at position 5 and Cys at position 14; and
Cys at position 7 and Cys at position 12.

23. The theta defensin peptide of claim 1, or a functional fragment thereof, having the amino acid sequence:

Xaa1-Xaa2-Xaa9-Xaa4-Xaa10-Xaa1-Xaa11-Xaa4-Xaa4-Xaa1-Xaa1-
5 Xaa12-Xaa4-Xaa13-Xaa1-Xaa14-Xaa7-Xaa8,

wherein: Xaa1 independently is an aliphatic amino acid;

Xaa2 is an aromatic amino acid;

Xaa4 independently is Arg or Lys;

Xaa7 is Thr or Ser;

10 Xaa8 is Arg or Lys;

Xaa9 is Glu, Asp, Lys or Ser;

Xaa10 is Glu, Asp, Lys or Ser;

Xaa11 is Glu, Asp, Lys or Ser;

Xaa12 is Glu, Asp, Lys or Ser;

15 Xaa13 is Glu, Asp, Lys or Ser;

Xaa14 is Glu, Asp, Lys or Ser.

24. The theta defensin of claim 23, wherein an intrachain crosslink is formed between two amino acids selected from the group consisting of

20 Xaa9 and Xaa14;

Xaa10 and Xaa13; and

Xaa11 and Xaa12.

25 25. The theta defensin of claim 24, wherein said crosslink is selected from the group consisting of lactam and lactone.

26. The theta defensin of claim 1, said theta defensin having antimicrobial activity against a microorganism selected from the group consisting of a gram positive bacterium, a gram negative bacterium, a yeast and a fungus.

27. The theta defensin of claim 26, wherein said microorganism is selected from the group consisting of *Staphylococcus sp.*, *Listeria sp.*, *Escherichia sp.*, *Salmonella sp.*, *Candida sp.*, and *Cryptococcus sp.*

28. The theta defensin of claim 27, wherein said microorganism is selected from the group consisting of *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, *Candida albicans*, and *Cryptococcus neoformans*.

29. The theta defensin of claim 1, said theta defensin having antimicrobial activity against a protozoan.

30. The theta defensin of claim 29, wherein said protozoan is selected from the group consisting of *Giardia sp.* and *Acanthamoeba sp.*

31. The theta defensin of claim 1, said theta defensin having antimicrobial activity against a virus.

32. The theta defensin of claim 31, wherein said virus is human immunodeficiency virus-1.

33. A pharmaceutical composition, comprising the theta defensin of claim 1 and a pharmaceutically acceptable carrier.

34. The pharmaceutical composition of claim 33, which is associated with a liposome.

35. The pharmaceutical composition of claim 33, which is associated with a non-liposome lipid complex.

36. An antibody that specifically binds the theta defensin peptide of claim 1.

37. The antibody of claim 36, wherein said theta defensin peptide has the amino acid sequence:

10 Gly-Phe-Cys-Arg-Cys-Leu-Cys-Arg-Arg-Gly-Val-Cys-Arg-Cys-Ile-Cys-Thr-Arg (SEQ ID NO:1).

38. The antibody of claim 36, which is a monoclonal antibody.

39. An isolated nucleic acid molecule encoding
15 a theta defensin, or a functional fragment thereof, said theta defensin having antimicrobial activity.

40. The nucleic acid molecule of claim 39,
said theta defensin peptide comprising the amino acid
sequence:

Xaa1-Xaa2-Xaa3-Xaa4-Xaa5-Xaa1-Xaa6-Xaa4-Xaa4-Xaa1-Xaa1-
5 Xaa6-Xaa4-Xaa5-Xaa1-Xaa3-Xaa7-Xaa8,

wherein: Xaa1 independently is Gly, Ile, Leu, Val or
Ala;

Xaa2 is Phe, Trp or Tyr;

Xaa3 is Cys or Trp;

10 Xaa4 independently is Arg or Lys;

Xaa5 is Cys or Trp;

Xaa6 is Cys or Trp;

Xaa7 is Thr or Ser; and

Xaa8 is Arg or Lys,

15 or a nucleic acid molecule complementary thereto.

41. The nucleic acid molecule of claim 40,
wherein said theta defensin peptide has the amino acid
sequence:

Gly-Phe-Cys-Arg-Cys-Leu-Cys-Arg-Arg-Gly-Val-Cys-Arg-Cys-
20 Ile-Cys-Thr-Arg (SEQ ID NO:1).

42. The nucleic acid molecule of claim 39,
said nucleic acid molecule comprising the RTD1a
nucleotide sequence referenced as SEQ ID NO:17.

43. The nucleic acid molecule of claim 39,
25 said nucleic acid molecule comprising the RTD1b
nucleotide sequence referenced as SEQ ID NO:19.

44. The nucleic acid molecule of claim 39,
said nucleic acid molecule comprising the RTD1a
nucleotide sequence referenced as SEQ ID NO:13.

45. The nucleic acid molecule of claim 39,
5 said nucleic acid molecule comprising the RTD1b
nucleotide sequence referenced as SEQ ID NO:15.

46. The nucleic acid molecule of claim 39,
said nucleic acid molecule comprising the RTD1a
nucleotide sequence referenced as SEQ ID NO:24.

10 47. The nucleic acid molecule of claim 39,
said nucleic acid molecule comprising the RTD1b
nucleotide sequence referenced as SEQ ID NO:25.

48. The nucleic acid molecule of claim 39,
said nucleic acid molecule comprising the human theta
15 defensin nucleotide sequence referenced as SEQ ID NO:28.

49. A nucleotide sequence that hybridizes
under moderately stringent conditions to the nucleic acid
molecule of claim 39.

50. A vector encoding a theta defensin, said
20 vector comprising an expression element operationally
linked to a nucleotide sequence encoding a theta defensin
peptide, said nucleotide sequence comprising the nucleic
acid molecule of claim 39.

51. A method of reducing or inhibiting growth or survival of a microorganism in an environment capable of sustaining the growth or survival of the microorganism, comprising administering an effective
5 amount of a theta defensin to said environment, thereby reducing or inhibiting the growth or survival of the microorganism.

52. The method of claim 51, which has antimicrobial activity against a microorganism selected
10 from the group consisting of a gram positive bacterium, a gram negative bacterium, a yeast and a fungus.

53. The method of claim 52, wherein said microorganism is selected from the group consisting of
15 *Staphylococcus sp.*, *Listeria sp.*, *Escherichia sp.*, *Salmonella sp.*, *Candida sp.*, and *Cryptococcus sp.*

54. The method of claim 53, wherein said microorganism is selected from the group consisting of
20 *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, *Candida albicans*, and *Cryptococcus neoformans*.

55. The method of claim 51, which has antimicrobial activity against a protozoan.

56. The method of claim 55, wherein said protozoan is selected from the group consisting of
25 *Giardia sp.* and *Acanthamoeba sp.*

57. The method of claim 51, which has antimicrobial activity against a virus.

58. The method of claim 57, wherein said virus is human immunodeficiency virus-1.

59. The method of claim 51, wherein said environment is a food or food product.

5 60. The method of claim 51, wherein said environment is a solution.

61. The method of claim 60, wherein said solution is a contact lens solution.

10 62. The method of claim 60, wherein said solution is an eye wash solution.

63. The method of claim 51, wherein said environment is an inanimate object comprising a surface.

64. The method of claim 51, wherein said environment is a mammal.

15 65. The method of claim 51, wherein said administration is topical.

66. The method of claim 51, wherein said administration is by injection.

20 67. The method of claim 51, wherein said administration is oral.

68. A method of preparing a cyclic peptide comprising,

(a) synthesizing a linear peptide of an amino acid sequence corresponding to the amino acid sequence of theta defensin,

(b) forming one or more crosslink bonds within said linear peptide, and

(c) cyclizing said peptide by linking the carboxyl and amino termini to form a cyclic peptide.

69. The method of claim 68, wherein said crosslink is selected from the group consisting of disulfide, lanthionine, lactam and lactone.

70. The method of claim 68, wherein the cysteine residues used in said linear peptide are in a pre-formed activated ester form.

71. The method of claim 69, wherein the carboxyl terminus and amino terminus of said linear peptide are each approximately the same number of amino acids from the nearest cysteine.

72. The method of claim 71, wherein said disulfide bonds are formed by oxidation.

73. The method of claim 72, wherein said cyclizing is done with ethylenediaminecarbodiimide and N-hydroxybenzotriazole in a solvent.

74. The method of claim 73, where approximately 60 equivalents of ethylenediaminecarbodiimide and approximately 20 equivalents of N-hydroxybenzotriazole are used.

5 75. The method of claim 74, where the dimethylsulfoxide is the solvent.

76. The method of claim 68, wherein said cyclized peptide is resistant to exo-peptidases.

10 77. A method of enhancing protease resistance of a peptide, comprising synthesizing a peptide, wherein the amino-terminal amino acid and carboxyl-terminal amino acid of said peptide are positioned by intrachain crosslinks, whereby a peptide bond is formed between said amino-terminal and carboxyl-terminal amino acids.

15 78. A method of expressing a theta defensin, comprising

(a) administering the vector of claim 50 to a cell; and

20 (b) expressing said encoded theta defensin peptides, wherein said peptides form a theta defensin.

79. The method of claim 78, wherein said vector encodes two theta defensin peptides.

25 80. The method of claim 78, wherein a second vector encoding a second theta defensin peptide is administered to said cell.

81. An isolated peptide ligase, comprising an activity capable of forming a peptide bond between two polypeptides.

82. The isolated peptide ligase of claim 69,
5 wherein said polypeptides are theta defensin peptides.

83. A method of reducing or inhibiting growth or survival of a microorganism in an individual, comprising administering a molecule, wherein said molecule increases expression of a theta defensin.